THE FINE STRUCTURE OF THE PERITROPHIC MEMBRANES OF CERTAIN INSECTS

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The structure and formation of the peritrophic membrane (p.m.) of many insects have been studied extensively (Wigglesworth, 1950; Richards, 1951). Although the need for more detailed knowledge of the fine structure of the membrane was apparent, the results of early attempts to use electron microscopy were not encouraging (Richards and Korda, 1948). Later, Huber and Haasser (1950) reported some details of the p.m. of *Dixippus;* they found it to be a (p. 397) "more or less regular network, probably fibrous, with a thin film stretched across the holes of the network." Shortly afterwards, networks with meshes approximately 0.2 μ across and composed of fine fibrils were found electron microscopically by Lagermalm, Philip and Gralén (1950) in the excreta of the clothes moth larvae (*Tineola bisselliella*). These networks originated from the insect, and not from the wool which formed its food; it was not proved that they originated from the peritrophic membrane, although this seemed likely because of the similarity between these networks and those described by Huber and Haasser. Huber (1950) reported further details of the structure of the p.m. in *Periplaneta orientalis, Tenebrio molitor* and *Bombyx mori.* In all except the last the characteristic networks were found.

Cross-sections of the peritrophic membrane examined in the light microscope show it to consist of several loosely adhering layers, each roughly 0.5 μ to 1 μ thick. Since the networks are much thinner than this, it appears that each thicker layer can be further separated into thinner layers.

There are said to be two methods of formation of peritrophic membranes. In one, the membrane originates solely from specialized cells at the anterior end of the midgut and in the other the membrane is said to be produced by delamination of a series of concentric lamellae from the surface of all the cells of the midgut. The first type is said to occur in Diptera and Dermaptera and the second in certain Orthoptera, and in hymenopterous and coleopterous larvae (Wigglesworth, 1950). There is, however, still much uncertainty about the origin of the membranes and the nature of the secretory processes producing them. It is possible that a study of the fine structure will help to clarify this problem and to throw light on the physiology of absorption of food and passage of enzymes.

The present investigation began with the chance discovery in saliva of *Periplaneta* of membranes similar to those described by Huber and Haasser and by Lagermalm, Philip and Gralén. This present paper deals with the fine structure of these membranes and those of other insects, and the problem of the formation of the membranes.

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Experimental

A. Sources of material

Most of the material was obtained from the cockroach *Periplaneta americana* (L.) which proved such a satisfactory source that all the illustrations are derived from this insect. However, it is desirable in the first place to determine whether conclusions drawn from a study of this species have a general application; and secondly, since two types of p.m. corresponding to two methods of formation have been described, to examine and compare membranes of these two alleged types. This survey has not yet been carried very far, but the p.ms. of two other species have been found to contain membranes of the same general construction as those of *Periplaneta;* these are *Locusta migratoria* (L.) and *Galleria mellonella* (L.). In the following species membranes were not discovered: the dermapteran, *Titanolabis colossea* (Dohrn); the larva of the blowfly, *Lucilia cuprina* (Wied.); larva of the cabbage white butterfly, *Pieris rapae* (L.), of the dermestid beetle *Attagenus piceus* (Oliv.), and the worker honeybee, *Apis mellifera* (L.). A negative finding, it is emphasized, must be regarded with reserve since an element of chance enters into a search of this nature, and a more extensive search may lead to a positive result. It is not, therefore, possible to state with certainty yet whether two varieties of membrane exist.

Because networks had been previously found in *Tineola* excreta (Lagermalm, Philip and Gralén, 1950), several examinations were made of aqueous suspensions of this material without finding other than traces of fibrils.

B. Preparation of material for electron microscopy

Two procedures were suitable for the preparation of material from *Periplaneta* for examination in the electron microscope: (1) stimulation of the insect to cause saliva to exude from the mouth; and (2) dissection of the peritrophic membrane followed by its disintegration in water.

1. Saliva. The oral exudate from *Periplaneta* consists of a clear fluid which was dried directly on the ordinary specimen screen. The dried screen was washed several times with water in order to remove soluble salts, etc. This technique gave quite clean fields and about one quarter of all screens so prepared yielded membranes.

Following the discovery of membranes in *Periplaneta* possible sites of origin were considered, although their similarity to the structures described by Huber and Haasser (1950) made the peritrophic membrane seem the most probable. Proteinase activity could not be detected in the saliva as might have been expected if the contents of the midgut, which are certainly regurgitated into the crop (Day and Powning, 1949), had been able to reach the mouth parts. The possibility, therefore, that the chitinous intima of the salivary reservoirs or crop might be the source of the membranes was explored. Membranes were searched for in crop scrapings and in the contents of the salivary reservoirs, without success. However, when p.ms. were dissected and treated according to the method described below, abundant membranes were immediately discovered. These were far more extensive than the fragments found in saliva and more details of their structure were apparent. It seems certain that the networks of the saliva were in fact fragments

of the p.m., thus indicating regurgitation of midgut contents and suggesting that the inability to detect proteinase activity in the saliva was due to deficiencies of the technique.

2. Dissection. Dissection of the p.m. proved the most reliable source of material. The membrane and contents were dissected from the midgut and allowed to stand in water at about 1–2° C. from a few hours to several days with occasional shaking. Finally, vigorous shaking dispersed the membrane into fragments. After the coarser fragments had settled, drops of the permanent suspension were dried on the specimen screens.

The material obtained from all of these methods of preparation has been subjected to the action of the digestive enzymes of the insect and this should be kept in mind when considering the micrographs.

C. Electron microscopy

The microscope used was an R.C.A. Type EMU, and standard methods of specimen mounting, shadowing and examination were employed. Most screens were shadowed with uranium before examination to facilitate discovery of the p.m. fragments and all the plates reproduced here illustrate such specimens. However, the general structural features of the membranes were visible without shadowing. Attempted staining with phosphomolybdic acid did not result in any differentiation of structure.

Results

Occasional thick sheets were noted in aqueous suspensions of dissected p.ms. These were too thick for examination with 50 K.V. electrons. It was assumed that such material represented the total p.m., *i.e.*, the layers visible as separate sheets in the light microscope, not resolved into components. At the edges of such sheets signs of fraying into thinner membranes and fibrils were sometimes seen.

In preparations derived from saliva or directly from the dissected membrane, the most commonly occurring and easily recognized constituent is the "*regular fibrillar network*" (Figs. 1 and 2), as this structure will be referred to in this paper. Other apparently different kinds of membranes were also found, although these may really be variants or defective forms of the regular networks. For example, a honeycomb-like formation ("*honeycomb network*") was occasionally observed (Fig. 3), and frequently superimposed upon the regular networks there occurred a distinct layer of irregularly arranged fibrils ("*irregular meshwork*") (Fig. 6). Adhering to most membranes and often interpenetrating the fibrillar components was found an amorphous ground substance which, since it seems to be partly removed by the procedures associated with preparation, may consist of a less resistant substance than the fibrils. The electron micrographs alone do not afford

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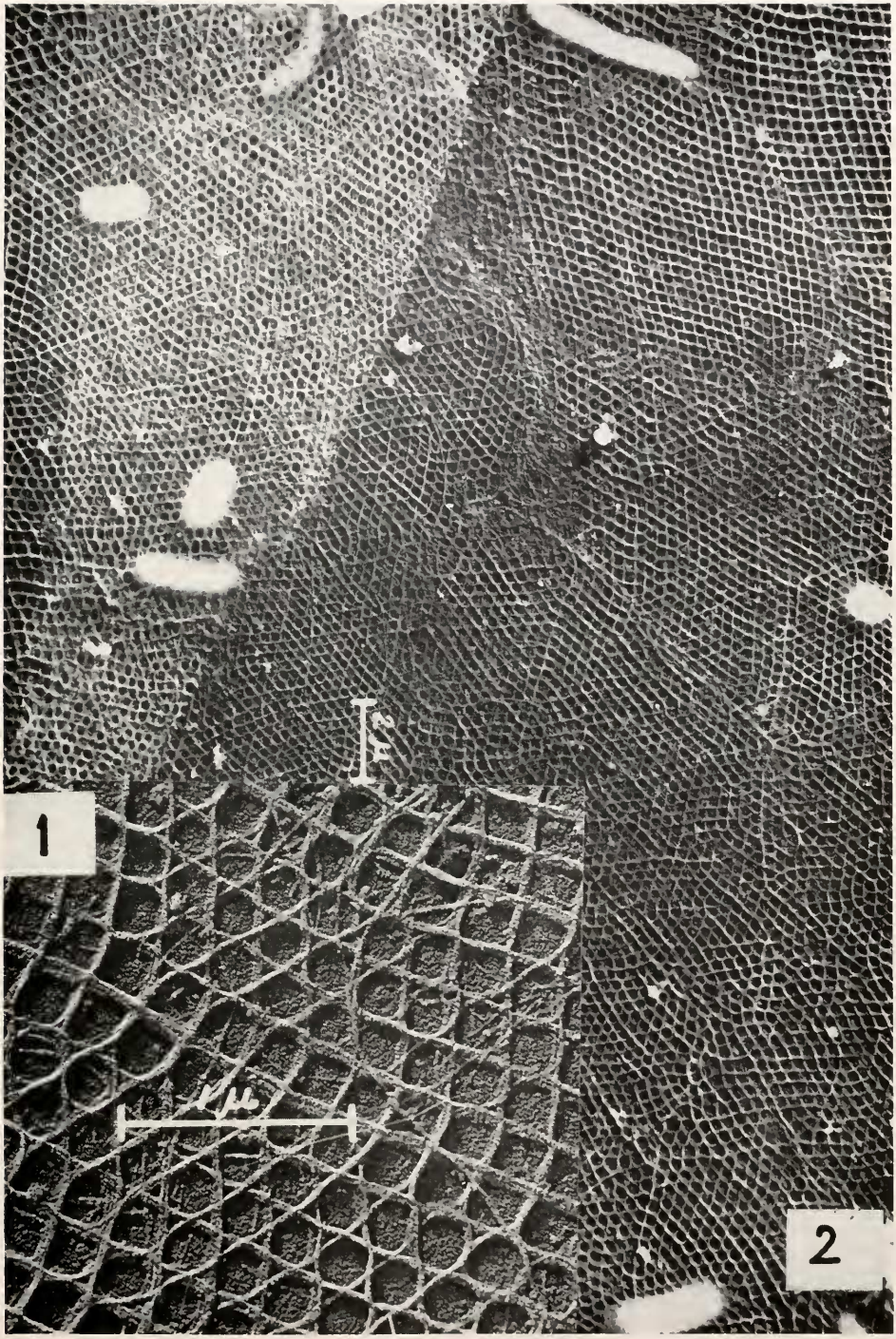
Figure 1. Fibrillar membrane from *Periplaneta*. Note areas with approximation to hexagonal symmetry and various types of defective structures. Each strand of the meshwork consists of several fine fibrils. Magnification: 32,000 ×.

Figure 2. Extensive area of the fibrillar membrane. Notice folds and structural irregularities. The size of the mesh may be compared directly with the bacteria also present. Magnification: 5200 ×.



FIGURES 1–2

definite proof that this amorphous substance is a structural constituent rather than a contaminant. However, since it seems to assist in maintaining the coherence of the total formation and is so thoroughly imbedded in the irregular meshwork, it will be assumed that it is a genuine component.

The regular fibrillar networks

The characteristics of these membranes can be appreciated from a study of Figures 1, 2 and 4. They are built from long straight strands or groups of fibrils of diameter probably less than 100 Å and of indefinite length. Ideally the structure tends towards hexagonal symmetry which can be seen in certain portions of the network shown in Figure 1. There are three sets of fibrils placed at 60° and forming triangular or hexagonal meshes (Fig. 8D). Commonly the structure is defective. A set of fibrils may be lacking, resulting in four-sided meshes (Fig. 8E), or the shape may be distorted. The number of separate fibrils composing a strand appears to vary, but is usually about four. Figure 4 illustrates fibrils protruding from the torn edge of a network. The size of the meshes is variable but on the average the parallel strands are about 0.15 μ to 0.2 μ apart. The networks were commonly found stretched flat across the supporting screens with occasional puckers or folds and frequently covered very considerable areas (*e.g.*, greater than 100 $\times$ 200 μ). The individual fibrils themselves seem to be structureless, although occasional ambiguous evidence of periodic nodulation was seen after shadowing.

The component fibrils of the strands are neither twisted together to form the strands nor regularly woven into the meshwork. They seem to form a purely random deposition as would result if a collection of straight fibers were sorted out upon an hexagonal array of pegs.

The irregular meshworks

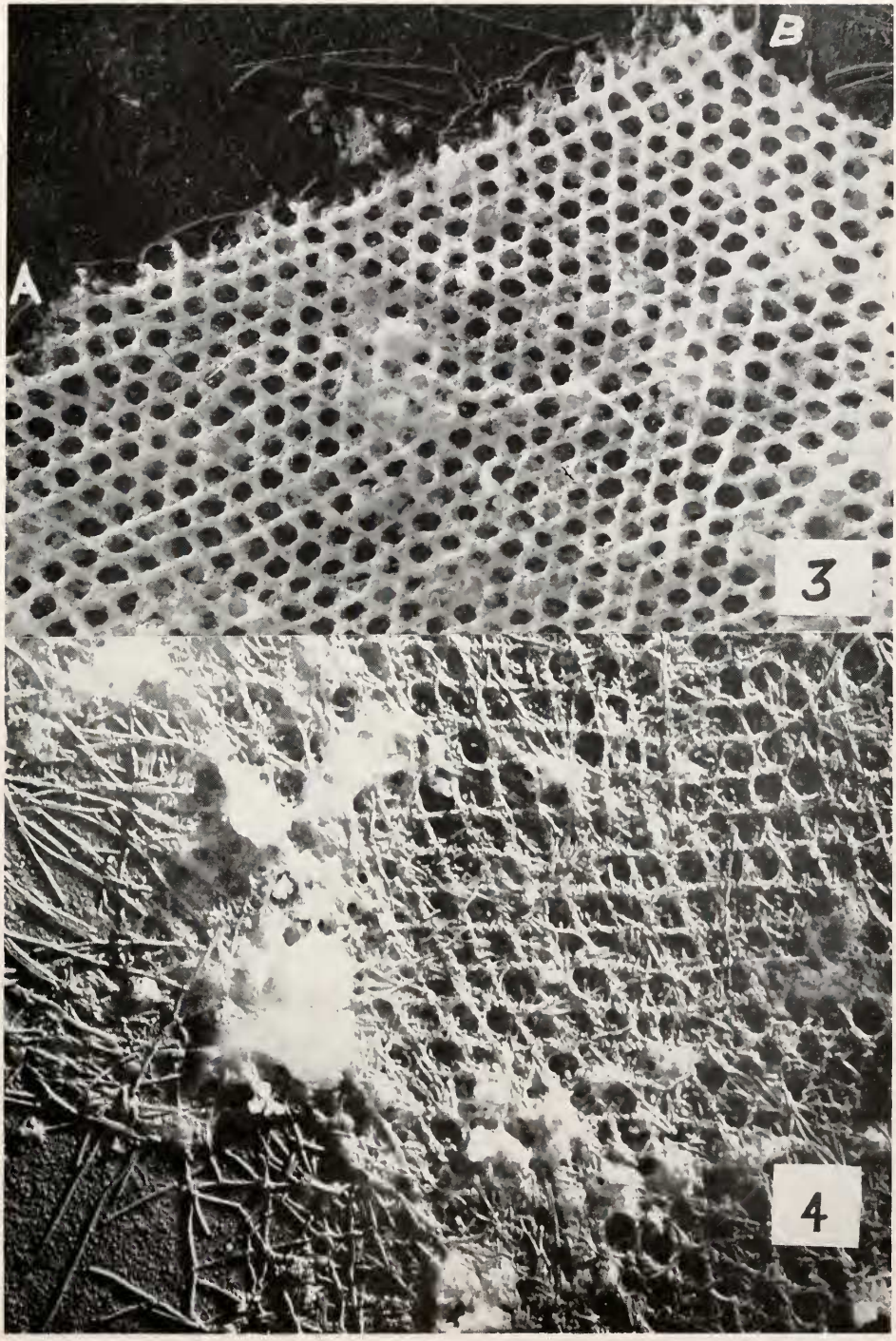
These formations seem to consist of fibrils similar in size to those forming the regular networks, but are less straight. They appear to be bent and felted together and imbedded in the amorphous ground substance which, by interpenetrating adjacent networks, very probably cements the whole laminated structure together. Occasional areas of ground substance lacking fibrils were noted. Some of the fibrils seem to continue directly into those composing the regular networks. This would suggest that the two types of membranes are essentially similar but that the organization is defective in one case.

The honeycomb networks

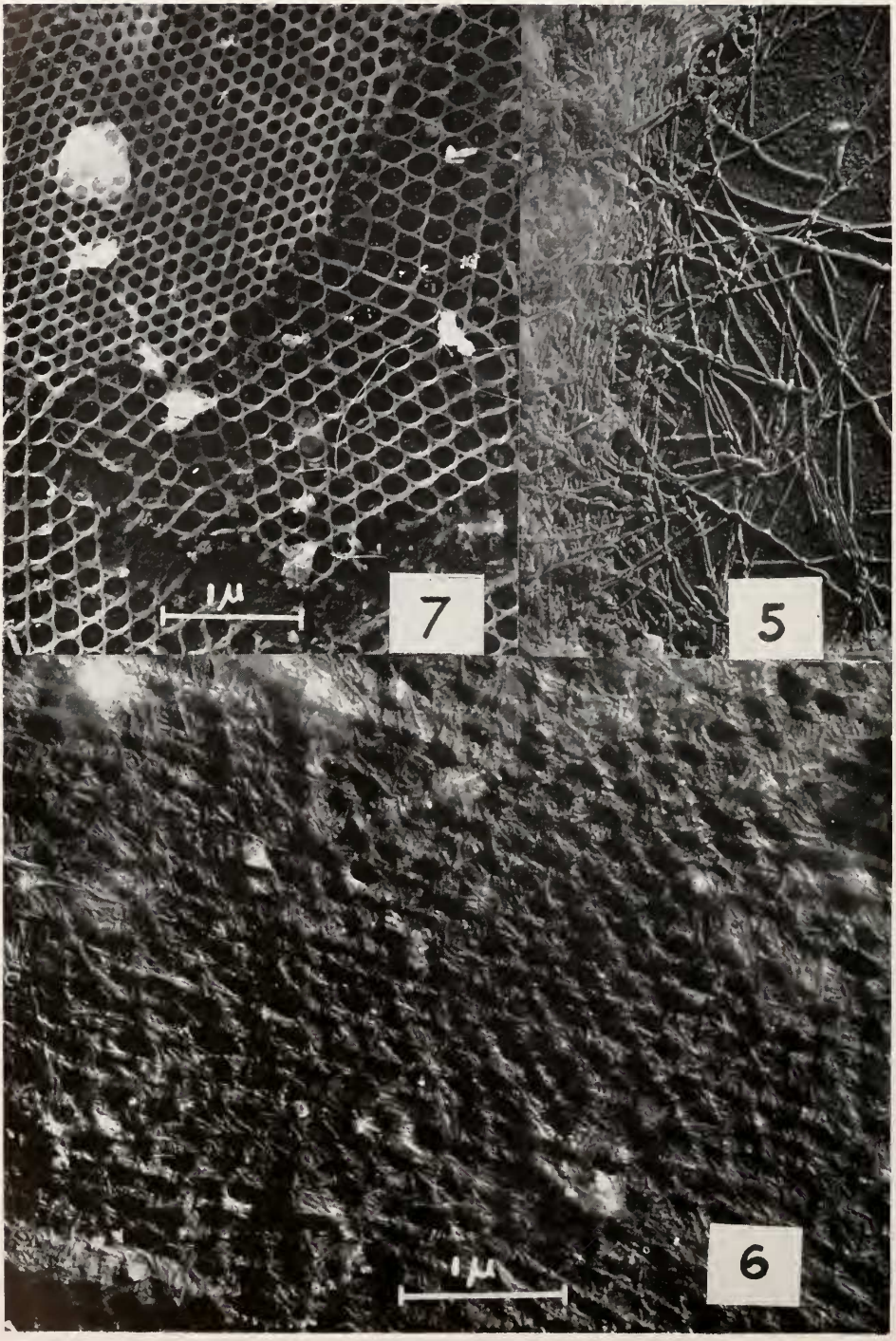
As can be seen from Figure 3 these networks present a distinctive appearance. In particular they tend to fracture along a straight line of holes, presenting a clean break distinct from that shown by the fibrillar networks (Fig. 4). They may, however, be of the same basic construction as the fibrillar network but consist of fewer

FIGURE 3. Example of "honeycomb network." Note straight edge at AB where membrane has broken. Magnification: 22,000 ×.

FIGURE 4. Edge of fibrillar network showing fraying into fibrils. Patches of ground substance containing fibrils may be seen. Magnification: 22,500 ×.



FIGURES 3–4



FIGURES 5, 6, 7

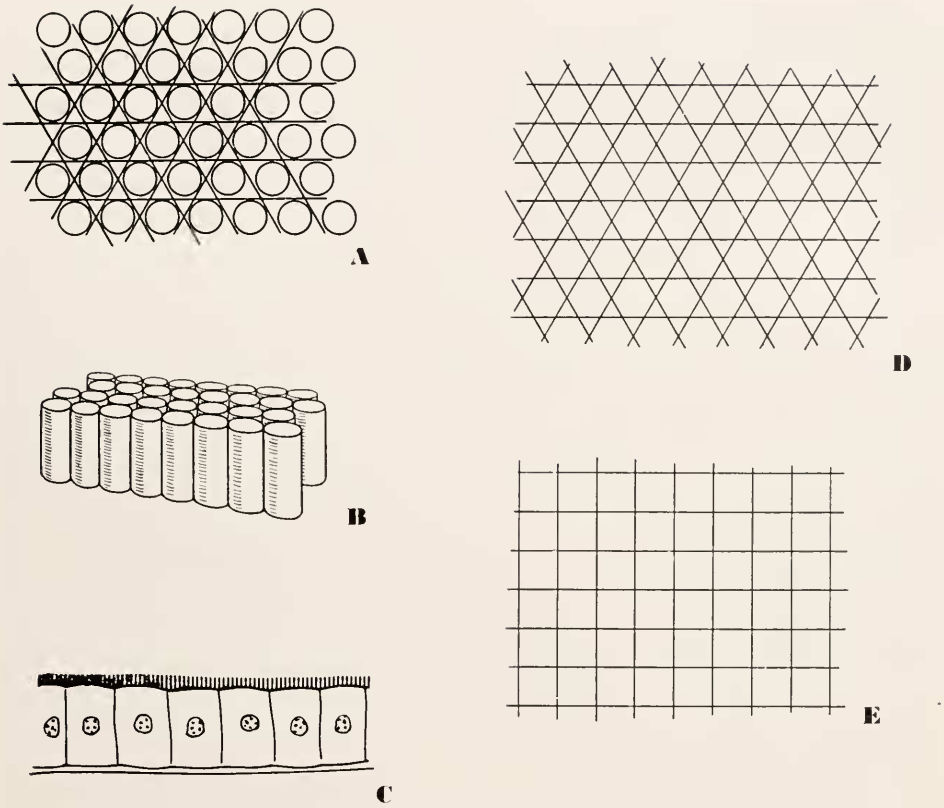


FIGURE 8. (A) Close-packed arrangement of spherical particles illustrating the formation of the hypothetical template surface having hexagonal symmetry. The way in which fibrils are deposited to form a hexagonal network (D) is indicated. (B) Similar surface of close-packed cylinders. (C) Diagram of striated border of midgut cells which may be formed by the aggregation into coarser fibrils of a finer structure such as shown at (B). (D) Regular fibrillar network as deposited on template of type (A). (E) Square network as deposited on a template of particles in square array formed by distortion of a close-packed array.

fibrils heavily encrusted with the amorphous ground substance. The holes may be either larger or smaller than the average size of the apertures in the regular networks.

DISCUSSION

A. Structure and function of peritrophic membrane

The coaxial lamellae composing the p.m. appear to possess a complex fine structure. Each layer seems to consist of a fine regular network associated with less

FIGURE 5. Example of imperfectly developed network. Magnification: 22,500 ×.

FIGURE 6. Fibrillar network with the irregularly ordered meshwork of fine fibrils partly superimposed. Notice presence of amorphous ground substance. Magnification: 22,500 ×.

FIGURE 7. A fibrillar network with a honeycomb structure lying above it. Magnification: 19,000 ×.

well defined layers of fibrils and amorphous ground substance. The fibrillar network is obviously that described by other authors (Huber and Haasser, 1950; Huber, 1950; Lagermalm, Philip and Gralén, 1950). The irregular meshwork is always found directly adhering to the fibrillar network, as in Figure 6. Here and there an actual continuity between the fibrils of the irregular mesh and the regular hexagonal network can be traced, suggesting that these two structures are formed from similar materials (fibrils and cement), but that in one case the regular order has failed to develop.

Histochemical studies have shown that both chitin and protein occur in the p.m. Although some doubt may exist as to the origin of the protein, it has been suggested that there is a chitinous framework with protein incorporated (Wigglesworth, 1950; Richards, 1951). This conception of the structure of the total membrane is in good agreement with the details now revealed by electron microscopy. It is most desirable to identify chemically the several components recognized, but unfortunately little in the nature of electron-histochemistry has been attempted up to the present time. In particular, since the average atomic weights of the atoms in chitin and in protein are of a similar order, a differentiation of these two materials on the basis of their electron-scattering power is not possible. Since chitin is usually fibrous, it seems not unlikely that the fibrillar component of the membranes is chitin and that the amorphous matrix or ground substance is protein. This conclusion was also reached by Huber (1950). In accord with the usual role of chitin as a skeletal substance, the fibrillar component may serve a mechanical function as reinforcement providing strength and coherence to the lamellar structure. The ideal arrangement of the fibrils, *i.e.*, three sets of parallel strands placed at 60° to each other, is mechanically adapted to the task of forming a tough membrane not readily torn in any direction.

The protein constituents of the complex, assuming these are represented by the continuous ground substance, might be expected to be the determining factor in the transport of solutes across the membrane. The networks, assuming these are more or less continuous, could of course act as coarser filters and prevent the passage of organisms, which might be able to digest and penetrate the protein component alone. The relative sizes of mesh aperture and bacteria may be seen in Figure 2, although the bacteria apparently entrapped here probably result from contamination occurring subsequent to dissection.

The broad features of membrane structure revealed in the p.m. may possibly prove to be of general occurrence, since the complex, fibrous chitin plus protein, is also found in insect cuticles (Richards, 1951) and closer examination of these may reveal fine fibrils of chitin embedded in an amorphous ground substance of a protein nature. Ribi (1951) has, in fact, reported the occurrence of fine chitinous fibrils in arthropod cuticle.

B. The formation of the peritrophic membrane

Certain aspects of the problem of the formation of the p.m. have been studied in sections by means of the light microscope. This work has been summarized by Wigglesworth (1950). Reference has been made above to the two methods of formation which have been suggested. A consideration of the fine structure of the membrane offers further data relevant to this problem. The features which seem to

be of significance are the lamellar structure and the two-dimensional order exhibited by certain of the networks. Both of these features suggest that these particular membranes are examples of exfoliation. In the first place a laminated structure can be readily imagined to form by the shedding of successive sheets from a secreting surface; and secondly, the fine structure of the individual networks could be plausibly explained if we suppose that the fibrils were laid down on a "template" possessing hexagonal symmetry.

It is worthwhile to speculate concerning the essential features of such a template. If the midgut cell surface possesses a pattern having hexagonal symmetry, the simplest method of achieving this is by the close packing of spheres or of circular rodlets. The outer surface could, for instance, be pictured as a close-packed array of spherical corpuscles (Fig. 8A) or of packed rodlets (Fig. 8B). The idea of packed rodlets is attractive because such a formation by aggregating to a coarser structure on fixation would simulate the striated border (diagrammed in Figure 8C) seen to fringe the midgut cells of most insects when viewed in the light microscope. It could then be assumed that the secretion, from which the fibrils separate spontaneously, deposits these fibrils directly in the "grooves" of the patterned surface (Figs. 8A and 8B). Those immediately adjacent to the patterned surface would be formed into the regular network; the excess of fibrils in the secretion more distant from the template surface would be less well ordered and form the mass of irregular fibrils embedded in the amorphous matrix which is found adhering to the regular networks. The matrix in which these fibrils are embedded is probably a second substance present in the secretion which has not the property of forming fibrils. In this way an intimately mixed chitin and protein (?) complex may result. The production of lamellae of structure alternating between a fibrillar texture and an amorphous texture would ultimately depend upon an alternating synthetic activity of the secreting cells which would determine the ratio of the precursors of these components in the secretion.

Since the networks extend over an area greater than that of a single midgut epithelial cell, the hypothetical hexagonal template must preserve the same orientation in adjacent cells. Picken, Pryor and Swann (1947), discussing the similar problem of orientation in cuticles, believe such a coordination unlikely and so are opposed to relating orientation to an underlying organization of the cell surface. However, having regard for the compressed packing of the cells of the columnar epithelium, it seems clear that the hexagonal shape of the cell ends themselves could determine the orientation of the close packing of the particles on their surfaces.

A suggestion similar in principle has been proposed to account for the origin of a two-dimensional array of fibrils observed on the earthworm cuticle by Reed and Rudall (1948) and for the formation of the radiolarian skeleton by Thompson (1942).

The authors are greatly indebted to Mr. J. Farrant for his invaluable suggestions and assistance with the electron microscopy; to Mr. D. F. Waterhouse for providing some of the material; to Mrs. N. Dewsnap for technical assistance; to Mr. L. A. Marshall for the preparation of the text figures; and to many colleagues for helpful discussions.

Summary

1. Some details of the fine structure of the peritrophic membrane of *Periplaneta* and of several other insects have been determined electron microscopically.

2. The membrane appears to be a complex structure of which the most characteristic and resistant component is a fibrillar network. A second component is a layer closely associated with the fibrillar network, consisting of unorganized fibrils embedded in an amorphous ground substance. The layers may be loosely associated and become separated on shaking the dissected membrane in water. The fibrils probably consist of chitin and the ground substance of protein.

3. The fibrillar network generally consists of three systems of parallel fibrillar strands placed at 60° to each other; thus it possesses hexagonal symmetry. A variety of defective arrangements, arising from mesh distortion, suppression of one set of fibrils and other grosser defects, may occur. The diameter of the fine fibrils composing the strands is about 100 Å and there may be several in each strand. The separate strands of a system of fibrils are about 0.15 to 0.2 μ apart. The fibrillar networks are well adapted to the formation of tough sheets, not readily torn.

4. Membranes of this nature were also found in *Locusta migratoria* and *Galleria mellonella*.

5. The nature of these membranes suggests that they are formed by delamination from a surface. This view of the mode of formation may account for the development of the fibrillar arrangement if it is assumed that the fine fibrils are deposited from a secretion onto a surface bearing an hexagonal pattern to act as a template.

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